For Research Use Only.
Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

RiboCluster Profiler™

RBP Antibody

Anti-U2AF1 pAb

CODE No. RN085PW

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified Ig from rabbit serum
FORMULATION PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED
Western blotting 1:1,000
Immunoprecipitation 5 µL/500 µL of cell extract from 1 x 10^7 cells

APPLICATION-UNDER EVALUATION
Immunocytochemistry 1:400

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, HepG2, Jurkat, 293T	NIH/3T3, WR19L	Rat1	CHO
Reactivity	+	+	+	+

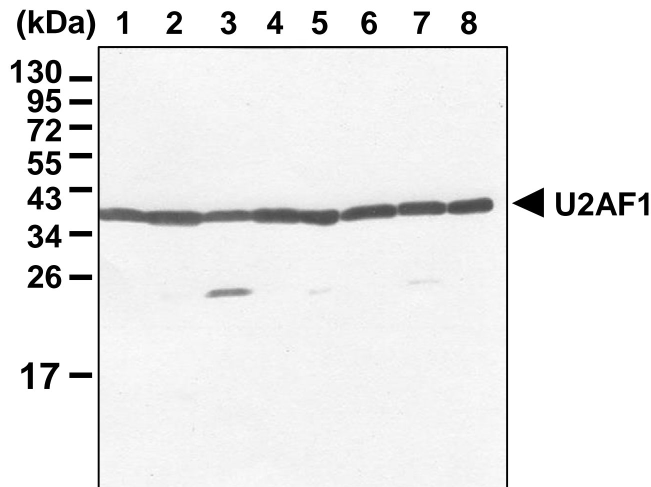
Entrez Gene ID 7307 (Human), 108121 (Mouse)

For more information, please visit our web site **https://ruo.mbl.co.jp/je/rip-assay/**

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (10 min. x 3).
8) Incubate the membrane with the 1:5,000 anti-rabbit IgG-HRP (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (10 min. x 3).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, NIH/3T3, Jurkat, HepG2, WR19L, Rat1, CHO)

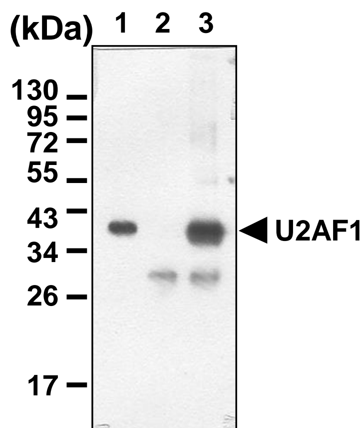


Western blot analysis of U2AF1
Lane 1: HeLa
Lane 2: 293T
Lane 3: NIH/3T3
Lane 4: Jurkat
Lane 5: HepG2
Lane 6: WR19L
Lane 7: Rat1
Lane 8: CHO
Immunoblotted with RN085PW

Immunoprecipitation

1) Wash 2 x 10^7 cells twice with PBS and resuspend them with 1 mL of ice-cold Lysis buffer [150 mM NaCl, 20 mM Tris-HCl (pH 8.0), 0.1% NP-40, 10 mM EDTA] containing appropriate protease inhibitors and 1.5 mM DTT. Vortex thoroughly, then incubate it on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and discard the supernatant.
3) Wash the pellet 3 times with PBS and resuspend them with 500 µL RIPA buffer, then sonicate briefly.
4) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another fresh tube.
5) Add 500 µL of ice-cold Lysis buffer into the supernatant. Mix by pipetting up and down.
6) Add 40 µL of 50% protein G agarose beads slurry resuspended in Lysis Buffer into the sample (prepared from step 5). Incubate it at 4°C with rotating for 1 hour.
7) Centrifuge the tube at 2,000 x g for 2 min. at 4°C and transfer the supernatant to another tube (precleared sample).
8) Mix 20 µL of 50% protein G agarose beads slurry resuspended in PBS with normal rabbit IgG or anti-U2AF1 pAb at the amount as suggested in the **APPLICATIONS,** then add 1 mL of Lysis Buffer into each tube. Incubate with gentle agitation for 1 hr. at 4°C.
9) Wash the beads once with 500 µL of ice-cold Lysis Buffer (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipette or without disturbing the beads.
10) Add 500 µL of nuclear extract (the sample from step 7), then incubate with gentle agitation for 3 hr. at 4°C.
11) Centrifuge the tube at 2,000 x g for 1 min. and discard the supernatant.
12) Resuspend the agarose with ice-cold Lysis buffer.
13) Centrifuge the tube at 2,000 x g for 1 min. and discard the supernatant.
14) Repeat steps 12)-13) 3 times.
15) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min., and centrifuge for 5 min. Use 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
16) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
17) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature, or overnight at 4°C.
18) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
19) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
20) Wash the membrane with PBS-T (10 min. x 3).
21) Incubate the membrane with the 1:1,000 Rabbit TrueBlot® anti-Rabbit IgG-HRP (Rockland, code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
22) Wash the membrane with PBS-T (10 min. x 3).
23) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
24) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

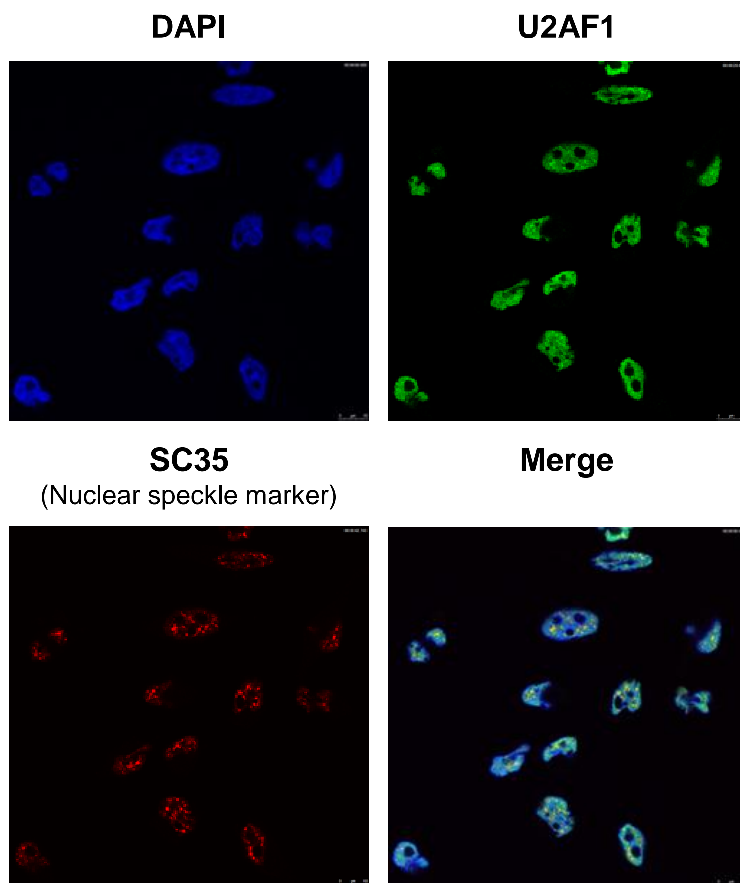
(Positive control for Immunoprecipitation; HeLa nuclear extract)



Immunoprecipitation of U2AF1 from HeLa

Lane 1: Input
Lane 1: IP with normal rabbit IgG
Lane 2: IP with RN085PW
Immunoblotted with RN085PW

Immunocytochemistry (Under evaluation)



Immunocytochemical detection of U2Af1 in HeLa Tet-Off cell

These data were provided by Dr. Akimitsu, The University of Tokyo.